S1.32 Intermediate positions of ATP on its trail to the binding pocket of subunit B and the high resolution structure of its neighbouring subunit F of the A_1A_O ATP synthase

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One focus of our laboratory is on the structural and mechanistical understanding of the ATP synthase from archaea. This class of enzyme is composed of ten subunits (A–K) in the stoichiometry of A_3:B_3:C:D:E:F:G:H_2:*a*:c_x. Combining solution X-ray scattering data and image processing of electron micrographs the low resolution structures of the, A_1 sector and/or the entire A_1A_O ATP synthase could be determined. The major subunits A and B of this complex are nucleotide-binding subunits. Previously, we determined a high resolution structure of subunit B in the absence of nucleotides at 1.5 Å. To get insight about the pathway of the nucleotide, moving towards the binding site, the B subunit mutant R416W was generated and co-crystallized with ATP. Here, we will show two intermediate positions of the nucleotide which could be trapped. There structures were determined to 2.0 Å and 3.3 Å resolution, showing the ATP molecule on its way to the actual binding pocket. In addition, the NMR solution structure of subunit F and its dynamic interaction with subunit B will be presented.

doi:10.1016/j.bbabio.2008.05.070

S1.33 Effect of C-terminal ε truncation on Pi regulation of the *E. coli* ATP-synthase

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The ε-subunit of the ATP-synthase is known as an endogenous inhibitor of the hydrolysis activity of the complex and undergoes drastic conformational changes between a non-inhibitory form (down-state) and an inhibitory form (up-state). It is thus conceivable that the α-helical C-terminal domain of this subunit is involved in regulating the enzyme. The aim of the present work was to explore this possibility by examining the regulatory behavior of a C-terminally truncated ε mutant. We have developed a low copy number expression vector carrying an extra copy of *uncC* with the aim of promoting normal levels of assembly of the mutated ATP-synthase complex. With this system, we have then compared ATP hydrolysis and proton pumping activity in membranes prepared from the WT and the $\varepsilon_{88\text{-stop}}$ truncated *E. coli* strains. Both strains showed well energized membranes. Noticeably, they showed a marked difference in their response to Pi inhibition of membrane-bound ATPase activity, which was largely lost in the truncated mutant. Since it has recently been shown that both ADP and Pi regulates the coupling degree of the pump, we are currently studying the effects of the C-terminal ε truncation on modulation of coupling efficiency by these ligands.

doi:10.1016/j.bbabio.2008.05.071

S1.34 What determines the size of the F_o rotor?

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The F_o motor of the ubiquitous F_1F_o F_1 ATP synthase, is composed of the subunits ab_2c_n. As a part of the rotor, the c subunits form an oligomeric ring of n subunits, each of which able to reversibly bind one ion during enzyme operation. Therefore, n equals the number of ion binding sites. As three molecules of ATP are produced in the F_1 motor per one revolution, the stoichiometry of the F_o-ring directly influences the ion to ATP ratio of the enzyme. It turned out that the stoichiometry of such rings are always constant in one species but vary among different species (n=10–15). Though, in most cases, the c ring stoichiometry seems to be defined only by the primary structure of the c subunit, the variation of c ring sizes could represent a strain specific adaptation to the particular physiological conditions. Recently, we have identified the stoichiometry of nine c rings from a group of cyanobacteria and despite their very high amino acid sequence identity, these c rings vary in their oligomeric state. By comparing these sequence differences with our structure of the c ring we could predict the specific regions of the c subunit critical for the ring assembly. The ongoing analysis of the oligomeric sizes of various c ring mutants addresses the contribution of the individual amino acid residues in the packing and assembly of F_o rotor.

doi:10.1016/j.bbabio.2008.05.072

S1.35 The rotor subunit interface of the ATP synthase from *Ilyobacter tartaricus*

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The strong interaction between the c_{11} ring and the γε complex forming the rotor of the *Ilyobacter tartaricus* ATP synthase was probed by Surface Plasmon Resonance spectroscopy and by *in vitro* reconstitution analysis. The assembly of the rotor was primarily dependent on the interaction of the c ring with the γ subunit while binding of the c ring to the free ε subunit was not observed. Mutagenesis of conserved amino acid residues at the interface of all three rotor components severely affected the rotor assembly. The quantitative assessment of interaction between the mutants of γε complex and c ring suggested that the assembly of the c_{11} ring with the γε complex is governed by interactions of low affinity between the polar loops of the c ring subunits and the bottom part of the γ subunit, and high affinity interactions, involving also the two residues γE204 and εH38 to stabilized the holo-c_{11}γε complex. Our results provide an explanation for the relative ease of dissociation and reconstitution of F_1F_o complexes described elsewhere and suggest that docking of the central stalk of the F_1 complex to the rotor ring of F_o might indeed be the last step in the assembly of the ATP synthase.

doi:10.1016/j.bbabio.2008.05.073